A New Method for ^{18}F-Labeling of Biochemical Molecules Using [^{18}F]Perfluoronitrobenzene and Its Application to ^{18}F-Labeling of Angiotensin II

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(Received September 13, 1996)

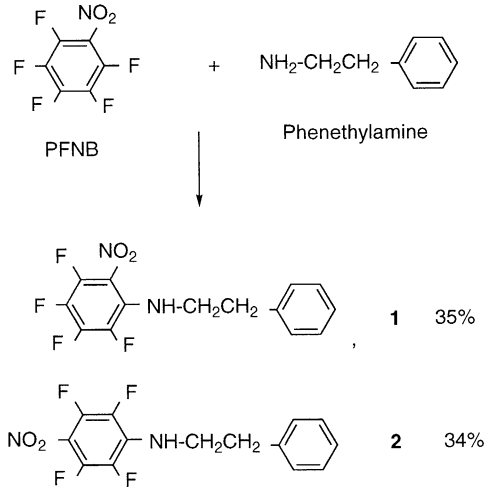
We propose a new method for labeling biochemical molecules with *fluorine*-18 (positron emitters) using [G-^{18}F]polyfluoronitrobenzene as the reagent. Perfluoronitrobenzene was easily labeled with $^{18}F^-$ by the ^{18}F-for-^{19}F exchange reaction giving [G-^{18}F]pentafluoronitrobenzene (PFNB, radiochemical yield 30-41%) and the [G-^{18}F]PFNB was applied successfully for ^{18}F-labeling of angiotensin II.

Positron labeled biochemical molecules (proteins, peptides and nucleic acids) have got nowadays wide biochemical applicabilities.[1] Among positron emitters, [^{18}F]fluorine has a longer half-life (110 min) and would be most suitable for studies which require a lengthy time to label biochemical targets. The weak positron energy (0.6 MeV, maximum range: 2.1 mm) of *fluorine*-18 brings about high resolution imaging data in positron emission tomography (PET), which is attractive and useful for biochemical and medical researches.[2] However, it is impossible to label biochemical molecules with *fluorine*-18 directly because of their instability at high temperature. In order to label them, several ^{18}F-labeling reagents have been designed and elaborated by methods of multi-step reactions from ^{18}F-fluoride.[3-6] These multi-step syntheses require complicated automated apparatuses, which suffer often machinery troubles. On the other hand, Kilbourn et al. synthesized [3-^{18}F]fluoro-5-nitrobenzoyl chloride from 3,5-dinitrobenzoyl chloride in a practical single-step synthesis[6,7] and applied it to ^{18}F-labeling of albumin.[5] Recently, some ^{18}F-labeled active esters ([^{18}F]fluoro-methylbenzoic acid N-hydroxysuccinimid ester) were synthesized by Lang et al.[7] However, it was not easy to obtain the precursors for them and, furthermore, the reagents might be unstable. We, too, reported a method for positron labeling of proteins by a photoaffinity labeling,[8] however, it was not suitable for small peptides because of random labeling.

To overcome these problems, we developed a new method which makes use of [^{18}F]di-or polyfluoronitrobenzenes. We proved that di- or polyfluoronitrobenzene derivatives [2,5-difluoronitrobenzene (DFNB), 2,4,6-trifluoronitrobenzene (TFNB), pentafluoronitrobenzene (PFNB)] are useful for labeling angiotensin II (AII) (Table 1), and the ^{18}F-labeling agents can be synthesized from [^{18}F]fluoride and the corresponding fluoronitrobenzenes using kryptofix®[2.2.2].

First, it was found that PFNB has two reactive sites (*para* and *ortho* positions to nitro). The main products of the reaction of PFNB (5 g, 23 mmol) with β-phenethylamine (1.2 g, 10 mmol) in the presence of aqueous triethylamine (TEA, 1% solution) for 1 hour at room temperature with stirring were 2-nitro-3,4,5,6-tetrafluorophenyl β-phenethyl amine (35%, **1**) and 4-nitro-2,3,5,6-tetrafluorophenyl β–phenethyl amine (34%, **2**) (Figure 1). **1** and **2** were stable in 6 N hydrochloric acid for 2 hours at 105 °C. The PFNB labeled aspartic acid, which gave only one colored spot on thin layer chromatography (TLC), but might be composit of two isomers as above, survived the acid-hydrolysis.

Figure 1.



Among the di-or polyfluoronitrobenzene derivatives investigated, PFNB was the most reactive to AII; PFNB (10 μmol) dissolved in 0.4 ml dimethyl sulfoxide (DMSO) was mixed with AII (200 μg) dissolved in 1% TEA at 37 °C, and free AII completely disappeared after 60 min' stirring at the same temperature (Table 1).

Synthesis of [^{18}F]PFNB.

[^{18}F]Fluoride was obtained by irradiating the [^{18}O]water (Isotec Inc., Ohio, USA) with 18 MeV proton beams generated by a cyclotron (Cypris HM-18, Sumitomo Heavy Industries, Ltd., Tokyo). The whole irradiated water (2.4 g) was recovered in a glassy-carbon vessel containing kryptofix[2.2.2] (15 mg, 40 μmol), 10 mM potassium carbonate (50 μl) and dry acetonitrile (0.5 ml), and then evaporated to dryness at 105 °C under low pressure (below 15 mmHg). Then, an anhydrous acetonitrile (0.5 ml) solution of PFNB (0.5 mmol) was added to the residue and the mixture heated at 105 °C for 30 min. No carrier [^{19}F]fluoride ion was added. The whole ^{18}F-labeling reaction mixture was injected onto a preparative HPLC column [Eluent : MeCN:10mM NaOH = 50 : 50 (v/v), Asahipak ODP-50 (Shoko Co., Ltd, Tokyo), φ 21.5 mm x 250 mm + guard column φ 21.5 mm x 100 mm, flow rate 12 ml/min, positron monitor : TCS-R81 (Aloka Co. Ltd., Tokyo)] to isolate the labeled compound (radiochemical yield 30-41%, radiochemical purity >99%, chemical purity >99%).

^{18}F-labeling of AII

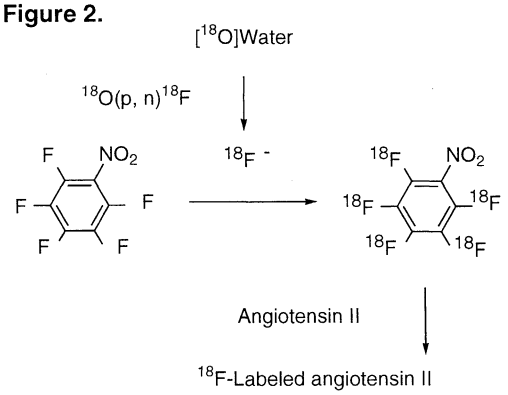
The [^{18}F]PFNB dissolved in DMSO mentioned above and AII (1 mg) dissolved in an aqueous solution (250 μl) of TEA (1%) were mixed and stirred at 37 °C for 60 min. The reaction mixture was injected onto a HPLC column [column: AG-100 (Shiseido Co. Ltd., Tokyo) φ 4.7 mm × 15 cm, A: acetonitrile : 0.1% hydrochloric acid (HCl) = 10 : 90, B:

Table 1. Reaction of angiotensin II with fluoronitrobenzene derivatives

Entry	Precursor (mmol/ml)	Labeling reagent (mmol/ml)	Reaction time (min)	Reaction temperature (°C)	Unreacted AII (%)
1	AII (0.2/200)	DFNB (10/400)	60	37	>90
2	AII (0.2/200)	TFNB (0.2/400)	120	r.t.	>90
3	AII (0.2/200)	PFNB (1/400)	60	37	not detected
4	AII (0.2/200)	PFNB (0.25/400)	2000	r.t.	30
5	AII (0.2/200)	PFNB (0.025/400)	2000	37	>90

r.t.: room temperature.

Figure 2.



acetonitrile : 0.1% hydrochloric acid= 70 : 30, gradient A→B in 40 min]. A radioactive peak (radiochemical yield 2%) was isolated and shown to be hypertensive in a rat experiment, however, the physiological activity was weak. The radiochemical purity and chemical purity were 99% by analytical HPLC [retention time 5.8 min (acetonitrile: 0.1% HCl= 25: 75)]. The total synthesis time was 120 min ([^{18}F]PFNB synthesis: 30 min, ^{18}F-labeling: 60 min, purification: 20 min). The label position in AII was estimated at the N-terminal amino group of aspartic acid by comparison of the TLC behavior of the hydrolysate and PFNB derivatives of eight amino acids composing AII. Moreover, if an other position was labeled, the biological activity may be reduced further.

A goal of the study on ^{18}F-labeling of peptides would be to develop a small ^{18}F-labeled reagent which has a reactive group to an amino group, and can be synthesized in single-step from a stable precursor available with ease.

This work was supported by a grant from the 'research and development programs for next-generation spinhead technologies' of the Japan Health Science Foundation. The authors are grateful to Dr. Y. Kawashima, President of the National Cardiovascular Center Japan, for supporting this work.

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